

Allowed claim 07/586, 535

PATENT  
454313-2335.1

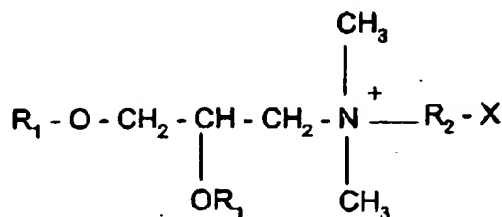
C3  
wt  
piglets are weighed once a week. Rectal temperatures are recorded on days 17, 21, 22, 24, 27, 29, 31, 34, 37, 41, 44. Day 44 fecal swabs are collected from each piglet for PCV-2 shedding. The virus is detected and quantified by quantitative PCR. Day 45 necropsies are performed and tissue samples are collected for virus isolation.--

Page 30, line one, please change "CLAIMS" to: --We Claim:--.

IN THE CLAIMS:

Please add the following new claims without prejudice, without admission, without surrender of subject matter, and without any intention of creating any estoppel as to equivalents:

12. (Amended) An immunogenic preparation comprising a complex of: at least one plasmid encoding and expressing *in vivo* in a porcine host an isolated nucleic acid molecule selected from the group consisting of open reading frame (ORF) 1 of porcine circovirus type II (PCV-2) and ORF2 of PCV-2; and, an adjuvant which comprises a cationic lipid of formula



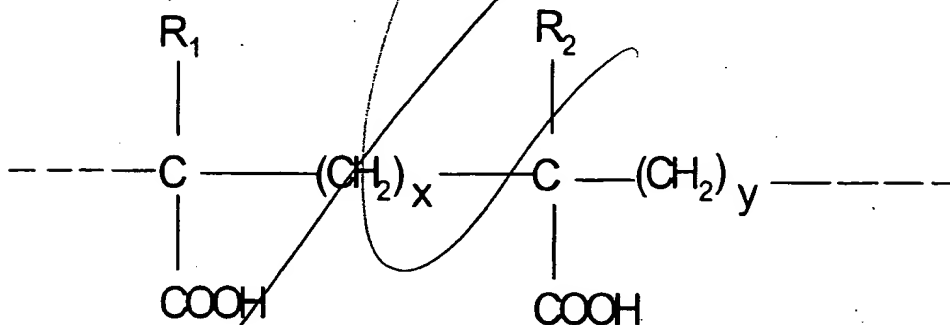
in which R<sub>1</sub> is a saturated or unsaturated linear aliphatic radical having from 12 to 18 carbon atoms, R<sub>2</sub> is aliphatic radical comprising from 2 to 3 carbon atoms, and X is an hydroxyl or amine group.

2 13. (Amended) An immunogenic preparation comprising at least one plasmid encoding and expressing *in vivo* in a porcine host an isolated nucleic acid molecule selected from the group consisting of open reading frame (ORF) 1 of porcine circovirus type II (PCV-2) and ORF2 of PCV-2; and, an adjuvant comprising a carbomer.

and expressing *in vivo* in a porcine host an isolated nucleic acid molecule selected from the group consisting of open reading frame (ORF) 1 of porcine circovirus type II (PCV-2), ORF2 of PCV-2, ORF1 of porcine circovirus type I (PCV-1) and ORF2 of PCV-1; and, an adjuvant comprising a carbomer.

3 14. (New) An immunogenic preparation comprising at least one plasmid encoding and expressing *in vivo* in a porcine host an isolated nucleic acid molecule selected from the group consisting of open reading frame (ORF) 1 of porcine circovirus type II (PCV-2), ORF2 of

PCV-2, ORF1 of porcine circovirus type I (PCV-1) and ORF2 of PCV-1; and, an adjuvant comprising a polymer having units of the formula:



in which:

$R_1$  and  $R_2$ , which are identical or different, represent H or  $\text{CH}_3$ ;

$x = 0$  or  $1$ ; and

$y = 1$  or  $2$ , with  $x + y = 2$ .

15. (New) The immunogenic preparation according to claim 12, wherein the cationic lipid is N-(2-hydroxyethyl)-N,N-dimethyl-2,3-bis(tetradecyloxy)-1-propanammonium (DMRIE).

16. (New) The immunogenic preparation according to claim 15, wherein DMRIE is coupled to a neutral lipid.

17. (New) The immunogenic preparation according to claim 16, wherein DMRIE is coupled to dioleoylphosphatidylethanolamine (DOPE).

18. (Amended) The immunogenic preparation according to any one of claims 12, 13, 15, 16 or 17 further comprising a porcine cytokine or a plasmid that encodes and expresses a porcine cytokine.

19. (New) The immunogenic preparation according to claim 18, wherein the porcine cytokine is GM-CSF.

20. (Twice Amended) The immunogenic preparation according to claim 12 or 13, further comprising a plasmid encoding and expressing an immunogen from a porcine pathogenic agent other than PCV-2.

*in addition*

10 21. (Amended) The immunogenic preparation according to any one of claims 12, 13, 15, or 16, wherein the preparation includes at least one plasmid that contains and expresses ORF1 of PCV-2.

e3  
11 22. (Amended) The immunogenic preparation according to any one of claims 12, 13, 15, or 16, wherein the preparation includes at least one plasmid that contains and expresses ORF2 of PCV-2.

12 23. (Amended) The immunogenic preparation according to any one of claims 12, 13, 15, or 16, wherein the preparation includes at least one plasmid that contains and expresses ORF1 and ORF2 of PCV-2.

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e3 24. (Twice Amended) The immunogenic preparation according to any one of claims 12, 13, 15, or 16, wherein the preparation includes at least two plasmids, one that contains and expresses ORF1 of PCV-2, and one that contains and expresses ORF2 of PCV-2.

~~25. (New) The immunogenic preparation according to any one of claims 12, 13, 14, 15, or 16, wherein the preparation includes at least one plasmid that contains and expresses ORF1 of PCV-1.~~

~~26. (New) The immunogenic preparation according to any one of claims 12, 13, 14, 15, or 16, wherein the preparation includes at least one plasmid that contains and expresses ORF2 of PCV-1.~~

~~27. (New) The immunogenic preparation according to claim 20, wherein the porcine pathogenic agent other than PCV-1 or PCV-2 is selected from the group consisting of Aujeszky's virus, porcine influenza virus, porcine reproductive and respiratory syndrome (PRRS), porcine parvovirus, hog cholera virus and *Actinobacillus pleuropneumoniae*.~~

14 28. (New) The immunogenic preparation of claim 17 wherein the DMR1E:DOPE molar ratio ranges from 95:5 to 5:95.

15 29. (New) The immunogenic preparation of claim 28 wherein the DMR1E:DOPE molar ratio is 1:1.

16 30. (New) The immunogenic preparation of claim 15 wherein the plasmid:DMR1E weight ratio ranges from 50:1 to 1:10.

17 31. (New) The immunogenic preparation of claim 15 wherein the plasmid:DMR1E weight ratio ranges from 10:1 to 1:5.

18 32. (New) The immunogenic preparation of claim 15 wherein the plasmid:DMRIE weight ratio ranges from 1:1 to 1:2.

19 33. (New) The immunogenic preparation of claim 17 wherein the plasmid:DMRIE-DOPE weight ratio ranges from 50:1 to 1:10.

20 34. (New) The immunogenic preparation of claim 17 wherein the plasmid:DMRIE-DOPE weight ratio ranges from 10:1 to 1:5.

21 35. (New) The immunogenic preparation of claim 17 wherein the plasmid:DMRIE-DOPE weight ratio ranges from 1:1 to 1:2.

C4  
cont 22 ~~36. (New) The immunogenic preparation of claim 14 wherein  $x=0$  and  $y=2$ .~~

~~37. (New) The immunogenic preparation of claim 18 wherein the preparation includes a plasmid that encodes and expresses a porcine cytokine which is GM-CSF.~~

38. (New) The immunogenic preparation of claim 27 wherein the immunogen from a porcine pathogenic agent other than PCV-2 or PCV-1 is selected from the group consisting of: glycoprotein gB of Aujeszky's virus, glycoprotein gD of Aujeszky's virus, porcine influenza virus H1N1 haemagglutinin, porcine influenza virus H1N1 nucleoprotein, porcine influenza virus H3N2 haemagglutinin, porcine influenza virus H3N2 nucleoprotein, the immunogen encoded by ORF5 of PRRS, the immunogen encoded by ORF3 of PRRS, the VP2 protein of the porcine parvovirus, the E1 protein of hog cholera virus, the E2 protein of the hog cholera virus, the immunogen encoded by the deleted apxI gene from *Actinobacillus pleuropneumoniae*, the immunogen encoded by the deleted apxII from *Actinobacillus pleuropneumoniae*, and the immunogen encoded by the deleted apxIII gene from *Actinobacillus pleuropneumoniae*.

23  
et 39. (Amended) A method for eliciting an immunogenic response in a porcine host against porcine circovirus comprising administering to the porcine host the immunogenic preparation of any one of claim 12, 13, 15 or 16.

Please cancel claims 14, 27, 36 and 38, without prejudice, without admission, without surrender of subject matter, and without any intention of creating any estoppel as to equivalents. 9/10/02

10/17/02

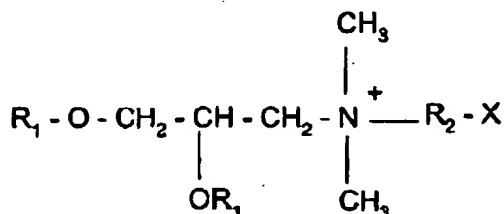
AMENDMENT

Kindly amend the application, without prejudice, without admission, without surrender of subject matter, and without any intention of creating any estoppel as to equivalents, as follows:

IN THE CLAIMS:

Please add the following new claims without prejudice, without admission, without surrender of subject matter, and without any intention of creating any estoppel as to equivalents:

24 40. (Amended) A method for enhancing a host immune response, in a porcine host, to a polypeptide encoded by open reading frame (ORF) 1 of porcine circovirus type II (PCV-2) or ORF2 of PCV-2, said method comprising administering to the porcine host at least one plasmid that encodes and expresses ORF1 of PCV-2 or ORF2 of PCV-2, wherein the plasmid is complexed with an adjuvant which comprises a cationic lipid of formula



in which R<sub>1</sub> is a saturated or unsaturated linear aliphatic radical having from 12 to 18 carbon atoms, R<sub>2</sub> is aliphatic radical comprising from 2 to 3 carbon atoms, and X is an hydroxyl or amine group.

25 41. (Amended) A method for enhancing a host immune response, in a porcine host, to a polypeptide encoded by open reading frame (ORF) 1 of porcine circovirus type II (PCV-2) or ORF2 of PCV-2, said method comprising administering to the porcine host at least one plasmid that encodes and expresses ORF1 of PCV-2 or ORF2 of PCV-2, and an adjuvant which comprises a carbomer.

porcine circovirus type I (PCV-1) or ORF2 of PCV-1 expressed *in vivo* in a porcine host by at least one plasmid that encodes and expresses *in vivo* in a porcine host the polypeptide, said method comprising administering the at least one plasmid with an adjuvant which comprises a carbomer.

26 42. (New) The method of claim 40 wherein the cationic lipid is N-(2-hydroxyethyl)-N,N-dimethyl-2,3-bis(tetradecyloxy)-1-propanammonium (DMRIE).

27 43. (New) The method of claim 42 wherein DMRIE is coupled to a neutral lipid.

28 44. (New) The method of claim 43 wherein DMRIE is coupled to  
dioleoylphosphatidylethanolamine (DOPE).

29 45. (New) The method of any one of claims 40 or 41 wherein the administering  
includes administering a porcine cytokine or a plasmid that encodes and expresses a porcine  
cytokine.

e<sub>1</sub> 30 46. (New) The method of claim 45 wherein the porcine cytokine is GM-CSF.

e<sub>2</sub> 31 47. (Amended) The method according to claim 40 or 41, wherein the administering  
includes administering a plasmid encoding and expressing an immunogen from a porcine  
pathogenic agent other than PCV-2.

32 48. (New) The method according to any one of claims 40 or 41 wherein the  
administering includes administering at least one plasmid that contains and expresses ORF1 of  
PCV-2.

33 49. (New) The method according to any one of claims 40 or 41 wherein the  
administering includes administering at least one plasmid that contains and expresses ORF2 of  
PCV-2.

34 50. (New) The method according to any one of claims 40 or 41 wherein the  
administering includes administering at least one plasmid that contains and expresses ORF1 and  
ORF2 of PCV-2.

35 e<sub>3</sub> 51. (Amended) The method according to any one of claims 40 or 41, wherein the  
administering includes administering at least two plasmids, one that contains and expresses  
ORF1 of PCV-2, and one that contains and expresses ORF2 of PCV-2.

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administering includes administering at least one plasmid that contains and expresses ORF2 of  
PCV-1.

36 54. (New) The method of claim 44 wherein the DMRIE:DOPE molar ratio ranges  
from 95:5 to 5:95.

37 55. (New) The method of claim 44 wherein the DMRIE:DOPE molar ratio is 1:1.

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- 38 56. (New) The method of claim 42 wherein the plasmid:DMRIE weight ratio ranges from 50:1 to 1:10.
- 39 57. (New) The method of claim 42 wherein the plasmid:DMRIE weight ratio ranges from 10:1 to 1:5.
- e1  
Cont 40 58. (New) The method of claim 42 wherein the plasmid:DMRIE weight ratio ranges from 1:1 to 1:2.
- 41 59. (New) The method of claim 44 wherein the plasmid:DMRIE-DOPE weight ratio ranges from 50:1 to 1:10.
- 42 60. (New) The method of claim 44 wherein the plasmid:DMRIE-DOPE weight ratio ranges from 10:1 to 1:5.
- 43 61. (New) The method of claim 44 wherein the plasmid:DMRIE-DOPE weight ratio ranges from 1:1 to 1:2.
- 44 62. (New) The method of claim 45 wherein the administering includes administering a plasmid that encodes and expresses a porcine cytokine which is GM-CSF.
- 45 63. (New) The method of claim 40 or 41 wherein the administering is intramuscularly.
- 46 64. (New) The method of claim 40 or 41 wherein the administering is intradermally.
- 47 65. (New) The method of claim 39 wherein the administering is intramuscularly.
- 48 66. (New) The method of claim 39 wherein the administering is intradermally.
67. (New) The immunogenic preparation of claim 12 or 13 which is for intramuscular administration.
68. (New) The immunogenic preparation of claim 12 or 13 which is for intradermal administration.--

Please amend the claims without prejudice, without admission, without surrender of subject matter, and without any intention of creating any estoppel as to equivalents as follows: